Maximum Likelihood Jukes-Cantor Triplets: Analytic Solutions

Benny Chor[*]   Michael D. Hendy[†]   Sagi Snir[‡]

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Abstract

Complex systems of polynomial equations have to be set up and solved algebraically in order to obtain analytic solutions for maximum likelihood on phylogenetic trees. This has restricted the types of systems previously resolved to the simplest models - three and four taxa under a molecular clock, with just *two state* characters. In this work we give, for the first time, analytic solutions for a family of trees with *four* state characters, like normal DNA or RNA. The model of substitution we use is the Jukes-Cantor model, and the trees are on three taxa under molecular clock, namely *rooted triplets*.

We employ a number of approaches and tools to solve this system: Spectral methods (Hadamard conjugation), a new representation of variables (the *path-set spectrum*), and algebraic geometry tools (the resultant of two polynomials). All these, combined with heavy application of computer algebra packages (Maple), let us derive the desired solution.

Key words: Maximum likelihood, phylogenetic trees, Jukes-Cantor, Hadamard conjugation, analytical solutions, symbolic algebra.

1   Introduction

Maximum likelihood (ML) is increasingly used as an optimality criterion for selecting evolutionary trees (Felsenstein, 1981), but finding the global optimum is a hard computational task, which led to using mostly *numeric* methods. So far, *analytic solutions* have been derived only for the simplest models (Yang, 2000; Chor et al., 2001, 2003) – three and four taxa under a molecular clock, with just *two state* characters (Neyman, 1971). In this work we present, for the first time, analytic solutions for a general family of trees with *four* state characters, like normal DNA or RNA. The model of substitution we use is the Jukes-Cantor model (Jukes and Cantor, 1969), where all substitutions have the same rate. The trees we deal with are three taxa ones, namely *rooted triplets* (see Figure 1).

[*]School of Computer Science, Tel-Aviv University, Tel-Aviv 39040 Israel. Research supported by ISF grant 418/00. `benny@cs.tau.ac.il`

[†]Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Palmerston North, New Zealand. `m.hendy@massey.ac.nz`

[‡]**Corresponding author.** Computer Science dept., Technion, Haifa 32000, Israel. `ssagi@cs.technion.ac.il`
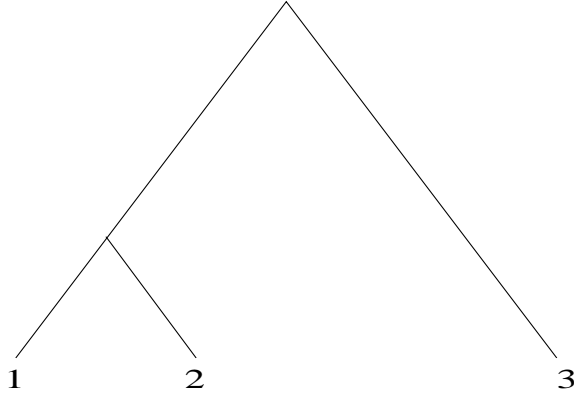
Figure 1: A rooted tree over 3 species.

The change from two to four character states incurs a major increase in the complexity of the underlying algebraic system. Like previous works, our starting point is to present the general maximum likelihood problem on phylogenetic trees as a constrained optimization problem. This gives rise to a complex system of polynomial equations, and the goal is to solve them. The complexity of this system prevents any manual solution, so we relied heavily on Maple, a symbolic mathematical system. However, even with Maple, a simple attempt to solve the system (*e.g.* just typing `solve`) fails, and additional tools are required. Spectral analysis (Hendy and Penny, 1993; Hendy et al., 1994) uses Hadamard conjugation to express the expected frequencies of site patterns among sequences as a function of an evolutionary tree T and a model of sequence evolution along the edges of T. As in previous works, we used the Hadamard conjugation as a basic building block in our method of solution. However, it turns out that the specific way we represent the system, which is determined by the choice of variables, plays a crucial role in the ability to solve it. In previous works (Chor et al., 2001, 2003), the variables in the polynomials were based on the *expected sequence spectrum* (Hendy and Penny, 1993). This representation leads to a system with two polynomials of degrees 11 and 10. This system is too complex to resolve with the available analytic and computational tools. By employing a different set of variables, based on the *path-set spectrum*, we were able to arrive at a simpler system of two polynomials whose degrees are 10 and 8. To finesse the construction, we use algebraic geometry combined with Maple. Specifically, we now compute the resultant of the two polynomials, which yields a single, degree 11 polynomial in one variable. The root(s) of this polynomial yield the desired ML solution. We remark that similar results to the ones shown here, were obtained by Hosten, Khetan and Sturmfels (Hosten et al., 2004), however by using somewhat different techniques.

It is evident that the currently available heuristic methods, fail to predict the correct tree even for small number of taxa. This is true not only in the presence of multiple ML points, but also in cases where the neighborhood of the (single) ML point is relatively flat. Therefore, a practical consequence of this work is the use of rooted triplets in supertree methods (constructing a big tree from small subtrees). For unrooted trees, the subtrees must have at least four leaves (*e.g. the tree from quartets* problem). For rooted trees, it is sufficient to amalgamate a set of rooted triplets (Aho et al., 1981). Our work enables such approaches to rely on rooted ML triplets based on four characters states rather than two.

Additionally, analytic solutions are capable to reveal properties of the maximum likelihood points that are not obtainable numerically. For small trees, our result can serve as a method for checking

the accuracy of the heuristic methods used in practice.

The remainder of this work is organized as following: In the next section we provide definitions and notations used throughout the rest of this work. In Section 3 we develop the identities and structures induced by the Jukes-Cantor model, while Section 4 develops maximum likelihood on phylogenetic trees and subsequently solves the system for the special case of three species under Jukes-Cantor and molecular clock. In Section 5 we show experimental results of applying our method on real genomic sequences, while Section 6 concludes with three open problems.

2   Definitions, Notations, and the Hadamard Conjugation

In this section we define the model of substitution we use, introduce useful notations, and briefly describe the Hadamard conjugation for the Kimura models of substitutions.

2.1   Definitions and Notations

We start with a tree labelling notation that will be useful for the rest of the work. We illustrate it for $n = 4$ taxa, but the definitions extend to any n. A *split* of the species is any partition of $\{1, 2, 3, 4\}$ into two disjoint subsets. We will identify each split by the subset which does *not* contain 4 (in general, n), so that for example the split $\{\{1, 2\}, \{3, 4\}\}$ is identified by the subset $\{1, 2\}$. For brevity, to label objects in a split, we concatenate the members of the split. Each edge e of a phylogenetic tree T induces a split of the taxa, which is the cut induced by removing e. We denote the edge e by the cut it induces. For instance the central edge of the tree $T = (12)(34)$ induces the split $\{\{1, 2\}, \{3, 4\}\}$, that is identified by the subset $\{1, 2\}$ and therefore this edge is denoted e_{12}. Thus the set of edges of T is $E(T) = \{e_1, e_2, e_{12}, e_3, e_{123}\}$ (see Figure 2).
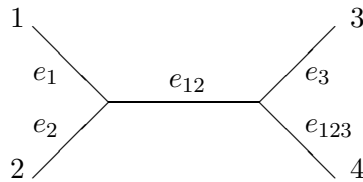

Figure 2: The tree $T = (12)(34)$ and its edges

Throughout the paper, we will index our vectors and matrices by a method denoted *subsets indexing*. We encode a subset of $\{1, 2, \ldots, n\}$ in an (n)-long binary number where the ith least significant bit $(i = 1, \ldots, n)$ is "1" if i is in the subset, and "0" otherwise. Using this representation, it is convenient to index the rows and columns of a matrix by subsets of $\{1, 2, \ldots, n\}$ in a lexicographically increasing order (i.e. $\phi, \{1\}, \{2\}, \{1, 2\}, \ldots, \{1, 2, \ldots, n\}$). Table 1 illustrates a matrix M indexed by subsets indexing over the set $\{1, 2\}$. The general element of M corresponding to subsets D and E, is denoted be $m_{D,E}$.

Extending the alphabet from two to four character states significantly increases the complexity of handling the data. In contrast to the binary case, as treated in previous works (Yang, 2000;

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Chor et al., 2001, 2003; Chor and Snir, 2004), where each site pattern in the sequence data induced a split, the four state site patterns induce a pair of splits. We will use the term *substitution pattern* to represent the substitutions to each taxon from a reference taxon. Let $X = \{1, 2, \cdots, n\}$ represent the set of taxa under study. We select n as a reference taxon and let $\bar{X} = X - \{n\}$ the set of non-referenced taxa. Consider a n-dimensional vector ν over the DNA alphabet, where each entry i correspond to a taxon i in X. The vector ν is called a character pattern. A substitution pattern is a $(n-1)$-dimensional vector of the substitution types $\nu_n \rightsquigarrow \nu_i$ for $i \in \bar{X}$.

For example, the character pattern $\begin{bmatrix} A \\ C \\ T \\ T \end{bmatrix}$ induces the substitution pattern $\begin{bmatrix} T \rightsquigarrow A \\ T \rightsquigarrow C \\ T \rightsquigarrow T \end{bmatrix}$.

Suppose a phylogenetic tree T over the set of taxa X is given, with substitution probabilities on each of its edges. Then, the probability of obtaining each substitution pattern is well defined. We remark that the number of substitution pattern is $\Sigma \times \Sigma^{n-1} = \Sigma^n$. For some popular models, the set of substitutions is substantially smaller than the general case.

The Kimura 3 substitution model (Kimura, 1983), is a model of symmetric nucleotide substitutions, implying convergence to equal base frequencies. In that model, Kimura proposed 3 classes of substitution: transitions (denoted α, $A \leftrightsquigarrow G$, $T \leftrightsquigarrow C$), type I transversions (denoted β, $A \leftrightsquigarrow T$, $G \leftrightsquigarrow C$) and type II transversions (denoted γ, $A \leftrightsquigarrow C$, $T \leftrightsquigarrow G$). Figure 3 illustrates these relations. We denote each of the substitution types with a pair of binary numbers: $t_\alpha = t_{01}$ for transitions, $t_\beta = t_{10}$, $t_\gamma = t_{11}$ for transversions and we write $t_\varepsilon = t_{00}$ for no substitution.

The number of substitution patterns with this coding is 4^{n-1} (for every taxon, the substitution $\nu_n \rightarrow \nu_i$ is either of type t_α, t_β, t_γ or t_ε).

We now define two subsets, $D, E \subseteq \bar{X}$, as follows: $D = \{i : \nu_n \rightarrow \nu_i \in \{t_\beta, t_\gamma\}\}$ and $E = \{i : \nu_n \rightarrow \nu_i \in \{t_\alpha, t_\gamma\}\}$. Since both D and E contain species with substitution type t_γ, they are *not* disjoint. To better understand this classification of the species into the sets D and E, we define an encoding of the character states as follows:

$$
\begin{aligned}
A &\rightarrow (1, 0) \\
C &\rightarrow (0, 1) \\
G &\rightarrow (1, 1) \\
T &\rightarrow (0, 0)
\end{aligned}
$$

	{} 00	{1} 01	{2} 10	{1,2} 11
{} 00				
{1} 01				
{2} 10				$m_{2,12}$
{1,2} 11				

Table 1: The matrix M indexed by split indexing. The element $m_{\{2\},\{12\}}$ is placed in the $(2,3)$ (binary $(10, 11)$) entry.
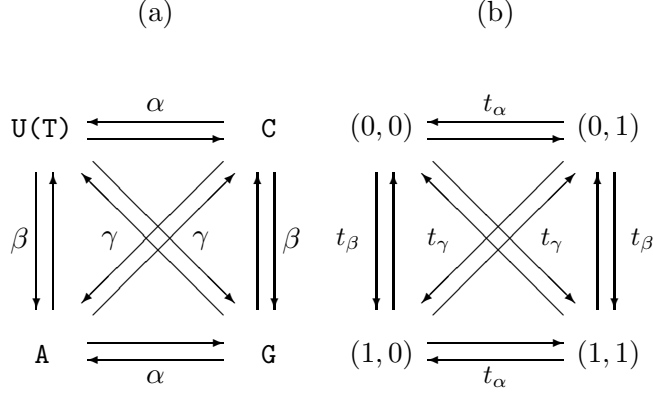
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Figure 3: (a) Kimura's 3–substitution model (K3ST). (b) Substitution types $t_\alpha = t_{01}$, $t_\beta = t_{10}$, $t_\gamma = t_{11}$ and $t_\epsilon = t_{00}$.

With this mapping, D contains the species i such that the first bit that encodes the state of i differs from the first bit that encodes the state of the reference species, n. The set E contains all species i such that the second bit that encodes the state of i differs from the second bit that encodes the state of species n. For example, suppose the character pattern ν is as follows:

species (i)	state (ν_i)	binary encoding	substitution	membership in D	membership in E
1	A	(1,0)	$t_\beta = (1,0)$	1	0
2	C	(0,1)	$t_\alpha = (0,1)$	0	1
3	G	(1,1)	$t_\gamma = (1,1)$	1	1
4	T	(0,0)			

We can view the set D (resp. E) as a split $\{D, \bar{X} \setminus D\}$ (resp. $\{E, \bar{X} \setminus E\}$). We encode every substitutions pattern by the two ordered splits (D, E) that define it. Let $s_{D,E}$ be the probability of obtaining the substitution pattern (D, E) on a tree. Both D and E range over all subsets of $\bar{X}$. Therefore it is natural to represent all probabilities $s_{D,E}$ in a matrix $S = [s_{D,E}]$, indexed by subsets indexing over $\bar{X} \times \bar{X}$. The rows are indexed by the split D and the columns by the split E. We call the matrix S the *expected sequence spectrum*. Since the number of splits over $\bar{X}$ is 2^{n-1}, S is a $2^{n-1} \times 2^{n-1}$ matrix.

For an edge e, let $q_e(\alpha)$, $q_e(\beta)$ and $q_e(\gamma)$ be the expected number of substitutions of type α, β, and γ, respectively. We call them the *edge length* parameters, so each edge is associated with three different "lengths", one per substitution type. Tree edges naturally correspond to splits. We extend the notion of edge lengths to splits that do not correspond to tree edges, by simply defining the length as zero: For a subset $D \subseteq \bar{X}$ such that $D \neq \emptyset$ and D is not an edge split, we set $q_D(\theta) = 0, (\theta \in \{\alpha, \beta, \gamma\})$. For $D = \emptyset$, we set $q_\emptyset(\theta) = -K(\theta)$ where $K(\theta)$ is the sum of all other $q_D(\theta)$ values. We define three vectors $\mathbf{q}_\theta$ for $\theta = \alpha, \beta, \gamma$ indexed by subsets indexing over $\bar{X}$ as follows: $\mathbf{q}_\theta = [q_D(\theta)|D \subseteq \bar{X}]$. Then $q_D(\theta) = 0$ implies there is no edge e_D in T (e.g. $\mathbf{q}_{13}(\theta)$, $\mathbf{q}_{23}(\theta)$ in T_{12}). Figure 4(a) shows the edge length spectra for the tree T_{12} on $n = 4$ taxa that was

$$\mathbf{q}_\alpha = \begin{bmatrix} -K(\alpha) \\ q_1(\alpha) \\ q_2(\alpha) \\ q_{12}(\alpha) \\ q_3(\alpha) \\ 0 \\ 0 \\ q_{123}(\alpha) \end{bmatrix}, \mathbf{q}_\beta = \begin{bmatrix} -K(\beta) \\ q_1(\beta) \\ q_2(\beta) \\ q_{12}(\beta) \\ q_3(\beta) \\ 0 \\ 0 \\ q_{123}(\beta) \end{bmatrix}, \mathbf{q}_\gamma = \begin{bmatrix} -K(\gamma) \\ q_1(\gamma) \\ q_2(\gamma) \\ q_{12}(\gamma) \\ q_3(\gamma) \\ 0 \\ 0 \\ q_{123}(\gamma) \end{bmatrix},$$

(a)

$$Q_T = \begin{bmatrix} -K & q_1(\alpha) & q_2(\alpha) & q_{12}(\alpha) & q_3(\alpha) & 0 & 0 & q_{123}(\alpha) \\ q_1(\beta) & q_1(\gamma) & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ q_2(\beta) & \cdot & q_2(\gamma) & \cdot & \cdot & \cdot & \cdot & \cdot \\ q_{12}(\beta) & \cdot & \cdot & q_{12}(\gamma) & \cdot & \cdot & \cdot & \cdot \\ q_3(\beta) & \cdot & \cdot & \cdot & q_3(\gamma) & \cdot & \cdot & \cdot \\ 0 & \cdot & \cdot & \cdot & \cdot & 0 & \cdot & \cdot \\ 0 & \cdot & \cdot & \cdot & \cdot & \cdot & 0 & \cdot \\ q_{123}(\beta) & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & q_{123}(\gamma) \end{bmatrix},$$

(b)

Figure 4: (a): Example edge length spectra for the tree T_{12}. (b): $Q = Q_{T_{12}}$

illustrated in Figure 2.

We will find it convenient to put these three vectors into a matrix $Q(= Q_T) = [q_{D,E}]$ of 2^{n-1} rows and columns indexed by subsets indexing over $\bar{X} \times \bar{X}$, with $q_{\emptyset,\emptyset} = -(K(\alpha) + K(\beta) + K(\gamma))$, and the remaining entries of $\mathbf{q}_\alpha$, $\mathbf{q}_\beta$ and $\mathbf{q}_\gamma$ becoming the leading row, column and main diagonal of Q respectively. All other entries of Q are set to 0. Figure 4(b) shows the matrix $Q = Q_{T_{12}}$ holding the vectors $\mathbf{q}_\alpha$, $\mathbf{q}_\beta$, $\mathbf{q}_\gamma$ from Figure 4(a). This means that for $D, E \subseteq \{1, 2, 3\}$, $Q_{\emptyset,D} = q_D(\alpha)$, $Q_{D,\emptyset} = q_D(\beta)$, $Q_{D,D} = q_D(\gamma)$, and for all other entries, $Q_{D,E} = 0$, except the first entry $Q_{\emptyset,\emptyset} = -(K(\alpha) + K(\beta) + K(\gamma))$. The entries indicated by "·" are all zero, and are zero for every tree. The entries indicated by "0" are zero for this specific tree T_{12}, but for different trees can be non-zero. The non-zero entries (in the leading row, column and main diagonal) should each be in the same component, and these identify the edge splits of T. For general trees on n taxa, the edge length spectra are vectors and square matrices of order 2^{n-1}.

2.2 Hadamard Conjugation

The Hadamard conjugation (Hendy and Penny, 1993; Hendy et al., 1994) is an invertible transformation that specifies a relation between the expected sequence spectrum S and the edge lengths spectra $\mathbf{q}(\theta)$ of the tree. In other words, the transformation links the probabilities of site substitutions on edges of an evolutionary tree T to the probabilities of obtaining each possible substitutions pattern. The Hadamard conjugation is applicable to a number of site substitution models: Ney-

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man 2 state model, Jukes–Cantor model (Jukes and Cantor, 1969), and Kimura 2ST and 3ST models (Kimura, 1983) (the last three models correspond to four states characters, such as DNA or RNA). For these models, the transformation yields a powerful tool which greatly simplifies and unifies the analysis of phylogenetic data, and in particular the analytical approach to ML.

Definition 1 *A* Hadamard matrix *of order ℓ is an $\ell \times \ell$ matrix A with ± 1 entries such that $A^t A = \ell I_\ell$.*

We will use a special family of Hadamard matrices, called Sylvester matrices in MacWilliams and Sloan (1977, p. 45), defined inductively for $n \geq 0$ by $H_0 = [1]$ and $H_{n+1} = \begin{bmatrix} H_n & H_n \\ H_n & -H_n \end{bmatrix}$. For example,

$$H_1 = \begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix} \text{ and } H_2 = \begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & -1 & 1 & -1 \\ 1 & 1 & -1 & -1 \\ 1 & -1 & -1 & 1 \end{bmatrix}.$$

H_n is indexed by subsets indexing over $\{1, \ldots, n\} \times \{1, \ldots, n\}$. Let $h_{D,E}$ be the general element of H_n. Then:

Observation 1 $h_{D,E} = (-1)^{|D \cap E|}$.

This implies that H_n is symmetric, namely $H_n^t = H_n$, and thus by the definition of Hadamard matrices $H_n^{-1} = \frac{1}{2^n} H_n$.

Proposition 1 *(Hendy and Penny 1993) Let T be a phylogenetic tree on n leaves with finite edge lengths ($q_e(\theta) < \infty$ for all $e \in E(T)$ and $\theta \in \{\alpha, \beta, \gamma\}$). Assume that sites mutate according to a symmetric substitution model, with equal rates across sites. Let S be the expected sequence spectrum and Q the edge length spectrum as was described above. Then*

$$S = S(Q) = H_{n-1}^{-1} \exp(H_{n-1}Q) , \tag{1}$$

where the exponentiation function $exp(x) = e^x$ is applied element wise to the matrix $R = H_{n-1}Q$.

This transformation is called the *Hadamard conjugation*.

Definition 2 *A matrix $\hat{\mathbf{S}} \in \mathbb{R}^{2^{n-1}} \times \mathbb{R}^{2^{n-1}}$ satisfying $\sum_{D,E \subseteq \{1,\ldots,n-1\}} \hat{\mathbf{S}}_{D,E} = 1$ and $H_{n-1}\hat{\mathbf{S}} > \mathbf{0}$ is called* conservative.

For conservative data $\hat{\mathbf{S}}$, the Hadamard conjugation is invertible, yielding :

$$\hat{\mathbf{Q}} = \hat{\mathbf{Q}}(\hat{\mathbf{S}}) = H_{n-1}^{-1} \ln(H_{n-1}\hat{\mathbf{S}})$$

site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	11	00	00	11	01	00	01	10	10	11	00	01	01	10	00	10
$\sigma_1 =$	C	C	A	T	C	A	A	A	C	G	T	G	T	G	A	C
	00	00	00	00	01	00	01	00	00	00	00	00	01	00	01	10
$\sigma_2 =$	A	C	A	G	C	A	A	T	G	T	T	A	T	C	T	C
	11	00	00	11	00	01	01	10	00	10	00	01	00	10	01	11
$\sigma_3 =$	C	C	A	T	T	G	A	A	G	A	T	G	C	G	T	T
$\sigma_4 =$	A	C	A	G	T	A	G	T	G	T	T	A	C	C	A	G

a

$$
F = \begin{bmatrix}
3 & 0 & 0 & 2 & 1 & 1 & 1 & 1 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
2 & \mathbf{1} & 0 & 0 & 0 & 2 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0
\end{bmatrix}
$$

b

Table 2: (a):Four aligned sequences with sixteen sites. (b): The corresponding observed sequence spectrum

where the ln function is applied element-wise to the matrix $H_{n-1}\hat{\mathbf{S}}$. We note that $\hat{\mathbf{Q}}$ is not necessarily the edge length spectrum of any tree. On the other hand, the expected sequence spectrum of any tree T is always conservative.

Consider now a set of n aligned homologous sequences $\sigma_1, \cdots, \sigma_n$, and denote this alignment as AL . We can view AL as a table where each column in this table induces a substitution pattern. Let $F_{D,E}$ be the frequency of the substitution pattern represented by the splits (D, E). The matrix $F = [F_{D,E}]$ is denoted as the *observed sequence spectrum* and is indexed analogously to the expected sequence spectrum matrix, S (that is, by subset indexing over $\bar{X} \times \bar{X}$).

Table 2(a) illustrates four sample DNA sequences with sixteen sites. σ_4 is the *reference* sequence, the pair of binary digits above each character of $\sigma_1, \cdots, \sigma_3$ is the substitution type to derive that character from the homologous character of σ_4. For example, the entry 11 above G at site 10 of σ_1 indicates that the substitution to this nucleotide from the corresponding T of the reference sequence σ_4 is of type t_γ. In (b), the frequencies of each of the site patterns from (a) are summarized in the observed sequence spectrum F. The rows of F are indexed by the first triple of the binary pairs, and the columns by the second, in the order $000, 001, 010, 011, 100, 101, 110, 111$. The site pattern of site 10 is represented by the pair $(101, 001)$ (or $D = \{1, 3\}$, $E = \{1\}$ alternatively) so the entry corresponding to this is in row 101 and column 001 of F. As this pattern occurs only at site 10, the entry in row 101 and column 001 of F is $\mathbf{1}$ (highlighted in **bold font**). We emphasize that the examples here refer to a tree on four leaves. The trees we solve for in the next sections have only *three* leaves.

3   Jukes–Cantor model for 3 sequences

The Jukes–Cantor model of evolution (Jukes and Cantor, 1969) is the simplest model for four states DNA evolution. The assumption in this model is that when a base changes, it has equal probabilities to change to each of the other three bases. This model can be derived from the more general Kimura $3-$ST model by setting, for each edge of T, each of the three edge length parameters equal to a common value, namely setting $q_e(\alpha) = q_e(\beta) = q_e(\gamma) = q_e$. We now look on the tree T on three taxa $\{0, 1, 2\}$ before determining where the root is. T has just one topology, the star with the three edges e_1, e_2 and e_{12}. For convenience we will write the edge length of e_{12} as q_3.

We now define several auxiliary matrices that will be useful in the sequel:

$$H = \begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & -1 & 1 & -1 \\ 1 & 1 & -1 & -1 \\ 1 & -1 & -1 & 1 \end{bmatrix}, J = \begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \end{bmatrix}, A_0 = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}, A_1 = \begin{bmatrix} 0 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$A_2 = \begin{bmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}, A_3 = \begin{bmatrix} 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 \end{bmatrix}, L = J - A_0 - A_1 - A_2 - A_3 = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 \\ 0 & 1 & 0 & 1 \\ 0 & 1 & 1 & 0 \end{bmatrix}.$$

The following identities relating these seven matrices, hold:

$$HJH = 16A_0, \tag{2}$$

$$HA_0H = J, \tag{3}$$

$$HA_1H = 4(A_0 + A_2) - J, \tag{4}$$

$$HA_2H = 4(A_0 + A_1) - J, \tag{5}$$

$$HA_3H = 4(A_0 + A_3) - J. \tag{6}$$

The edge–length spectrum of an arbitrary 3-tree can be expressed as the 4×4 matrix,

$$Q = \begin{bmatrix} -3(q_1 + q_2 + q_3) & q_1 & q_2 & q_3 \\ q_1 & q_1 & 0 & 0 \\ q_2 & 0 & q_2 & 0 \\ q_3 & 0 & 0 & q_3 \end{bmatrix} = q_1A_1 + q_2A_2 + q_3A_3 - 3(q_1 + q_2 + q_3)A_0.$$

Now, from Equations 2–6 we see

$$HQH = -4[(q_1 + q_3)A_1 + (q_2 + q_3)A_2 + (q_1 + q_2)A_3 + (q_1 + q_2 + q_3)L]$$

$$= -4 \begin{bmatrix} 0 & q_1 + q_3 & q_2 + q_3 & q_1 + q_2 \\ q_1 + q_3 & q_1 + q_3 & q_1 + q_2 + q_3 & q_1 + q_2 + q_3 \\ q_2 + q_3 & q_1 + q_2 + q_3 & q_2 + q_3 & q_1 + q_2 + q_3 \\ q_1 + q_2 & q_1 + q_2 + q_3 & q_1 + q_2 + q_3 & q_1 + q_2 \end{bmatrix},$$

so applying the exponential function to each element of the matrix HQH we obtain the so called path–set spectrum, R:

$$
\begin{aligned}
R & = \exp(HQH) \\
& = A_0 + x_1x_3A_1 + x_2x_3A_2 + x_1x_2A_3 + x_1x_2x_3L \\
& = \begin{bmatrix}
1 & x_1x_3 & x_2x_3 & x_1x_2 \\
x_1x_3 & x_1x_3 & x_1x_2x_3 & x_1x_2x_3 \\
x_2x_3 & x_1x_2x_3 & x_2x_3 & x_1x_2x_3 \\
x_1x_2 & x_1x_2x_3 & x_1x_2x_3 & x_1x_2
\end{bmatrix},
\end{aligned}
\tag{7}
$$

where

$$
x_i = e^{-4q_i}.
\tag{8}
$$

The x_i values can replace the q_i values as the defining parameters and are called the *path set variables*. The entries of R relate to the probabilities of differences between the end-points of paths in T.

By using Proposition 1, the expected sequence spectrum equals

$$
\begin{aligned}
S & = H^{-1}RH^{-1} \\
& = \frac{1}{16}[(1 + 3x_1x_2 + 3x_1x_3 + 3x_2x_3 + 6x_1x_2x_3)A_0 \\
& \quad +(1 - x_1x_2 - x_1x_3 + 3x_2x_3 - 2x_1x_2x_3)A_1 \\
& \quad +(1 - x_1x_2 + 3x_1x_3 - x_2x_3 - 2x_1x_2x_3)A_2 \\
& \quad +(1 + 3x_1x_2 - x_1x_3 - x_2x_3 - 2x_1x_2x_3)A_3 \\
& \quad +(1 - x_1x_2 - x_1x_3 - x_2x_3 + 2x_1x_2x_3)L] \\
& = \frac{1}{16}\begin{bmatrix}
a_0 & a_1 & a_2 & a_3 \\
a_1 & a_1 & a_4 & a_4 \\
a_2 & a_4 & a_2 & a_4 \\
a_3 & a_4 & a_4 & a_3
\end{bmatrix},
\end{aligned}
\tag{9}
\tag{10}
$$

where

$$
\begin{aligned}
a_0 & = (1 + 3x_1x_2 + 3x_1x_3 + 3x_2x_3 + 6x_1x_2x_3), \\
a_1 & = (1 - x_1x_2 - x_1x_3 + 3x_2x_3 - 2x_1x_2x_3), \\
a_2 & = (1 - x_1x_2 + 3x_1x_3 - x_2x_3 - 2x_1x_2x_3), \\
a_3 & = (1 + 3x_1x_2 - x_1x_3 - x_2x_3 - 2x_1x_2x_3), \\
a_4 & = (1 - x_1x_2 - x_1x_3 - x_2x_3 + 2x_1x_2x_3).
\end{aligned}
\tag{11}
$$

Thus we see that each expected sequence frequency takes one of the above values, which are functions of the three parameters x_1, x_2 and x_3.

4 Obtaining the Maximum Likelihood Solution

Given the observed frequencies, $F_{D,E}$, of each site pattern $(D, E) \subseteq \bar{X} \times \bar{X}$ (normalised so that $\sum_{D,E \subseteq \bar{X}} F_{D,E} = 1$), then for any expected sequence spectrum S of some tree T, the likelihood of obtaining those normalised frequencies is

$$
L(F|T, S) = \prod_{D,E \subseteq \hat{X}} S_{D,E}^{F_{D,E}}.
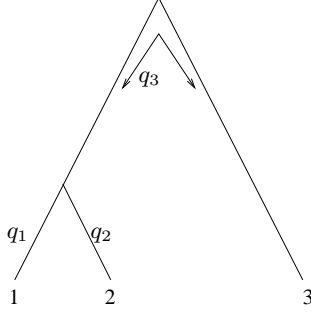\tag{12}
$$

Figure 5: A triplet tree under the molecular clock satisfies $q_1 = q_2$.

(It is convenient to use normalised frequencies as it simplifies the formulae later. This normal-isation scales $\log L$ by a constant factor, so does not affect the identity of the turning points.) Equation (10) gives identities among the pattern probabilities $S_{D,E}$ so grouping the common factors in equation (12) gives

$$L(F|T, S) = \prod_{j=0}^{4} a_j^{f_j}, \tag{13}$$

where

$$
\begin{aligned}
f_0 &= F_{\emptyset,\emptyset}, \\
f_1 &= F_{\emptyset,1} + F_{1,\emptyset} + F_{1,1}, \\
f_2 &= F_{\emptyset,2} + F_{2,\emptyset} + F_{2,2}, \\
f_3 &= F_{\emptyset,12} + F_{12,\emptyset} + F_{12,12}, \\
f_4 &= F_{1,2} + F_{1,12} + F_{2,1} + F_{2,12} + F_{12,1} + F_{12,2}.
\end{aligned}
$$

The expected sequence spectrum S can be expressed as a function of the three variables x_1, x_2 and x_3, so the values which maximise the likelihood L are obtained when the three partial derivatives, $\frac{\partial L}{\partial x_i}, (i = 1, 2, 3)$, are zero. In contrast to previous works (Chor et al., 2001; Chor and Snir, 2004; Chor et al., 2000, 2003) that operated in the space of the expected sequence variables, $S_{D,E}$, here we are operating in the space of the path-set variables. This eliminates the need to introduce the constraint of the ML points being on a "tree surface". By the chain rule, we get:

$$\frac{\partial L}{\partial x_i} = L \cdot \sum_{j=0}^{4} \frac{f_j}{a_j} \frac{\partial a_j}{\partial x_i} = 0, \text{ for } i = 1, 2, 3. \tag{14}$$

We require our ML tree to adhere to the molecular clock assumption, so a $((1,2),3)-$triplet tree under this assumption requires $q_1 = q_2 \leq q_3$ (see Figure 5) which implies $x_1 = x_2 \geq x_3$. In our analysis below we will explicitly impose the equality to find the turning points. The inequality will need to be tested on any potential solution, and if it were not satisfied, a maximum could be sought on the boundary of the valid tree domain, where $x_1 = x_2 = x_3$.

The constraint $x_1 = x_2$ implies $a_1 = a_2$, so by setting $f_{12} = f_1 + f_2$ and $a_{12} = a_1 = a_2$ we reduce the complexity of equation (14) to give two rational equations in two free variables and the parameters f_j:

$$\frac{\partial L}{\partial x_i} = L \cdot \left(\frac{f_0}{a_0} \frac{\partial a_0}{\partial x_i} + \frac{f_{12}}{a_{12}} \frac{\partial a_{12}}{\partial x_i} + \frac{f_3}{a_3} \frac{\partial a_3}{\partial x_i} + \frac{f_4}{a_4} \frac{\partial a_4}{\partial x_i} \right) = 0, \text{ for } i = 2, 3. \tag{15}$$

11

These simultaneously vanish when the two numerators, which are polynomials in x_2, x_3 and the parameters f_j, are both zero. We refer to these polynomial equations as E_1 and E_2.

We now show that the system of two resulting polynomials $\{E_1, E_2\}$ has only finitely many solutions, all of which we can find. The major tool used here is the *resultant* of two polynomials. Let $f(x) = \sum_{i=0}^{d} a_i x^i$ and $g(x) = \sum_{j=0}^{d} b_j x^j$ be two polynomials in one variable, x. The resultant of f and g, denoted $Res(f, g, x)$, is a polynomial in the coefficients a_i and b_j of f and g, which is 0 whenever f and g have a common zero. The coefficients can themselves be unknowns, or functions of other variables, in which case the resultant replaces the two polynomials f and g by a single polynomial in one fewer variable.

Computing the resultant is a classical technique for eliminating one variable from two equations. There is an elegant formula for computing it due to Sylvester, and another due to Bezout, which have been implemented in most computer algebra packages, such as `Maple`.

We can compute the resultant $ER = Res(E_1, E_2, x_3)$ of E_1 and E_2 with respect to x_3. This eliminates x_3 from the equations and yields a single polynomial ER, in just x_2 and the parameters. The polynomial ER has the form:

$$\begin{aligned}
ER \quad = \quad & k\, f_3\, f_{12}\, f_0\, x_2^{13} f_4\, (3\,x_2 + 1)\, (2\,x_2^2 + x_2 + 1)\, (3\,x_2^2 + 1)\, (3\,x_2^2 + 3\,x_2 + 2) \\
& (x_2 - 1)^2\, (x_2 + 1)^3 \cdot P_0
\end{aligned} \tag{16}$$

where P_0 is a degree 11 polynomial with 288 monomials and k is some big constant.

Theorem 1 *The turning points of L (equation 12) corresponding to realistic trees (namely, trees with positive edge lengths) are exactly the roots of P_0.*

Proof. The only term in ER except for P_0 (Equation 16) that admits positive real roots is the term $(x_2 - 1)$. However, by the definition of x_2, this root corresponds to $q_2 = 0$ which is not a realistic tree. ∎

Corollary 2 *The Jukes-Cantor triplet has a finite number of ML points.*

Proof. P_0 has at most 11 different solutions and for each such a solution we back substitute to obtain all the values of x_3. ∎

5 Results on Genomic Sequences

In order to evaluate our method, we tested it on real genomic sequences. We looked at the NK cell receptor D gene on human, mouse and rat (accession numbers AF260135, AF030313 and AF009511 respectively). We aligned the sequences using CLUSTALW (Thompson et al., 1994). Next, we computed the observed sequence spectrum, as explained in Section 2 and illustrated in Table 2. Three sequences have 16 site patterns and therefore the observed sequence spectrum is written in a 4-by-4 matrix. The resulting spectrum is shown in Table 3.

We calculated the maximum likelihood value for each of the three rooted trees under the model for the three species. As expected the ((rat,mouse),human) tree was maximal, with edge lengths $q_1 = q_2 = 0.0197$ to rat and mouse and $q_3 = 0.1061$ to human, giving the log likelihood $\ln L = -870.2$.

pattern frequency	00	01	10	11
00	424	18	18	80
01	1	7	2	2
10	7	4	4	4
11	27	1	2	40

Table 3: The observed sequence spectrum of NK cell receptor D gene of human, mouse and rat

We also calculated the maximum likelihood value for each of the three rooted trees for the beta actin gene for the three species guinea pig, goose and C elegans,(acc. numbers AF508792, M26111 and NM_076440 resp.) finding the ((guinea pig, goose), C elegans) tree maximal, with $q_1 = q_2 = 0.021819$ and $q_3 = 0.050188$ giving $\ln L = -1241.5$. Finally we calculated the maximum likelihood value for each of the three rooted trees for the histone gene of Drosophila melangoster, Hydra vulgaris and Human (acc numbers AY383571, AY383572 and NM_002107 resp.) finding the ((D. melangoster, H. vulgaris),Human) tree maximal, with $q_1 = q_2 = 0.001555$ and $q_3 = 0.012740$ with $\ln L = -86.835133$.

Each of the results above agree closely with the numerical values obtained using the popular phylogenetic reconstruction packages Phylip (Felsenstein, 1989) and PAUP* (Swofford, 1998) which use iterative methods to estimate the maxima.

6   Directions for Future Research

The progress made here brings up a number of open problems:

- Our ML solutions are derived from the roots of a univariate, degree 11 polynomial. This implies that the number of ML solutions is finite. It would be interesting to explore the question of *uniqueness* of the solution. If this is the case, it will most likely follow from the existence of a single solution corresponding to a realistic tree, as in (Chor et al., 2003).

- The Jukes-Cantor substitution model is the a special case of the family of Kimura substitution models. It would be interesting to further extend the result in this paper for the other models (two and three parameters) of the Kimura family.

- It would be interesting to extend these results to rooted trees with *four leaves* under JC model and a molecular clock. Here we have two different topologies – the fork and the comb (Chor et al., 2003). It is expected that such extension will face substantial technical difficulties.

References

Aho, A., Sagiv, Y., Szymanski, T., Ullman, J., 1981. Inferring a tree from lowest common ancestors with an application to the optimization of relational expressions. SIAM Journal of Computing 10 (3), 405–421.

Chor, B., Hendy, M., Holland, B., Penny, D., April 2000. Multiple maxima of likelihood in phylogenetic trees: An analytic approach. In: RECOMB2K, the Fourth Annual International Conference on Computational Molecular Biology. ACM, Tokyo, Japan, to appear.

Chor, B., Hendy, M., Penny, D., 2001. Analytic solutions for three taxon mlmc trees with variable rates across sites. In: WABI 2001.

Chor, B., Khetan, A., Snir, S., April 2003. Maximum likelihood on four taxa phylogenetic trees: Analytic solutions. In: Proceedings of the Seventh annual International Conference on Computational Molecular Biology (RECOMB). Berlin, Germany, pp. 76–83.

Chor, B., Snir, S., April 2004. Maximum likelihood molecular clock forks: Closed form analytic solutions.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Felsenstein, J., 1989. PHYLIP - phylogenetic inference package, (version 3.2). Cladistics 5, 164–166.

Hendy, M. D., Penny, D., 1993. Spectral analysis of phylogenetic data. J. Classif. 10, 5–24.

Hendy, M. D., Penny, D., Steel, M., 1994. Discrete fourier analysis for evolutionary trees. Proc. Natl. Acad. Sci. USA. 91, 3339–3343.

Hosten, S., Khetan, A., Sturmfels, B., August 2004. Solving the likelihood equations,. Http://front.math.ucdavis.edu/math.ST/0408270.

Jukes, T., Cantor, C., 1969. Evolution of protein molecules. In: Munro, H. (Ed.), Mammalian Protein Metabolism. Academic Press, New York, pp. 21–132.

Kimura, M., 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge.

Neyman, J., 1971. Molecular studies of evolution: A source of novel statistical problems. In: Gupta, S., Jackel, Y. (Eds.), Statistical Decision Theory and Related Topics. Academic Press, New York, pp. 1–27.

Swofford, D., 1998. PAUP*beta. Sinauer, Sunderland, Mass.

Thompson, J., Higgins, D., Gibson, T., 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalty and weight matrix choice. Nucleic Acids Research 22, 4673–4780.

Yang, Z., 2000. Complexity of the simplest phylogenetic estimation problem. Proc. R. Soc. Lond. B 267, 109–116.